REMARKS

The specification has been amended to correct typographical errors. The range "5541-5941" for the second oligonucleotide primer as used in paragraphs [0013], [0015], [0035] and claim 25 is supported in the specification as originally filed. One of ordinary skill in the art would know that "5491" originally recited in paragraph [0013] as "5541-5491" is a typographical mistake because the paragraph 0013 range goes from high to low, which is inconsistent with the remainder of the specification, and the range containing the typographical error excludes a preferred embodiment shown as IVS-R in Table 3. That 5491 of the range "5541-5491" should be "5541-5941" is supported by supported by paragraph [0035]. Thus, amendment of the specification to correct the range to "5541-5941" for the second oligonucleotide primer recited in paragraphs [0013], [0015], and [0035] raises no issue of new matter.

The value "5141" recited in paragraph [0035] is a typographical error and should instead read "5541" because 1) "5541" is correctly recited in paragraph [0013]; and 2) because the highest numbered position for the fragment is 5941, the 400 bp stretch of DNA described in paragraph [0035] must begin at position 5541. Thus, amendment of 5141 to 5541 in paragraph [0035] raises no issue of new matter.

Amendments to correct the position numbers in Tables 1 and 2 is supported by the GenBank accession entry. Thus, amendment of tables 1 and 2 raises no issue of new matter.

A formatting error occurred and the micro "μ" symbol was changed to " ⊠ " in various locations in paragraph [0065] and in Table 3. Support that the missing symbol is a "μ" is evidenced by the context of the text generally and the that fact that a "μ" symbol transferred to the final document correctly in paragraph [0065], line 4. Further, one of ordinary skill in the art would also know that individual amplification reactions are performed in microliter volumes. Support for "μ" for the stock solution concentrations in Table 3 is evident because the volumes are expressed in μl and the volumes and final concentrations would mathematically require the stock solutions to be in μM concentrations. Accordingly, the amendment of paragraph [0065] and Table 3 to replace " ⊠ " with a micro "μ" symbol raises no issue of new matter.

The specification has been amended to enter a new Sequence Listing, which includes sequences 1-8, and to insert new SEQ ID NO:8 into the appropriate pages of the specification. Also provided is a computer readable form (CRF) and certification. The new Sequence Listing supersedes all other sequence listings. As indicated in the accompanying statement under 37 C.F.R. §§ 1.821-1.825, the new Sequence Listing raises no issue of new matter as it merely represents sequences originally set forth in the application.

Claims 19-34 are pending in this application. Claims 1-18 and 35-36 have been cancelled without prejudice. Claims 19, 21, 22 and 25 have been amended to further define the invention. The amendments find support in the application and originally filed claims. For example, support for the amendments of steps i), ii) and iii) in claim 25 can be found at p. 4, paragraph [0013]; p. 9, paragraph [0035]; and p. 17, paragraph [0065]. Support for "deletion mutation" and "single base transition mutation" can be found, for example, at p. 6, paragraph [0022] and p. 13, paragraph [0050]. The deleted terms in claims 21 and 22 are due to typographical errors. "SEQ ID NO: 8" has been added to all instances in the claim which the MCOLN1 gene sequence is referred to further clarify the invention. Accordingly, the amendments of the claims and new claims raise no issue of new matter.

Information Disclosure Statement

The Examiner's assertion that the Information Disclosure Statement contains a non-relevant reference is acknowledged. Citation A1 listed as U.S. Patent No. 6,028,290 should instead be U.S. Patent No. 6,028,190. Attached herewith is a supplemental IDS for the intended reference.

Sequence Listing

The Examiner's assertion that specification attempts to incorporate a GenBank Accession entry as a reference which is not appropriate under 37 C.F.R. § 1.57 is acknowledged. Applicant has provided the sequence for the MCOLN1 gene as SEQ ID NO: 8 and has amended the specification and complied with Sequence Listing rules. A Rule 132 declaration provided by

Rosa Cheuk Kim also is provided to support that SEQ ID NO: 8 is identical to the sequence that was in GenBank AF287270 at the time the application was filed. Accordingly, withdrawal of the objection is respectfully requested.

Specification

The specification has been amended to correct various typographical errors in the description of the invention, specifically nucleotide positions, as noted by the Examiner. Accordingly, withdrawal of the various objections is respectfully requested.

Claim Objections

The Examiner's assertion that claim 21 contains the extra word "and" is acknowledged. The extra word has been deleted from claim 21. Applicant respectfully requests reconsideration and withdrawal of the objection.

The Examiner's assertion that claim 22 contains the extra phrase "and the probe in ii) is" is acknowledged. The extra phrase has been deleted from claim 22. Applicant respectfully requests reconsideration and withdrawal of the objection.

Rejection under 35 U.S.C. § 112

The Examiner's assertion that the term "the MCOLN1 gene" as used in claims 19-34 is allegedly indefinite is acknowledged. As noted, the specification has been amended to include the MCOLN1 gene sequence as SEQ ID NO: 8. Thus Applicant respectfully requests reconsideration and withdrawal of the rejection.

The Examiner's assertion that claims 25-34 are allegedly incomplete due to the omission of essential steps is acknowledged. Claim 25 has been amended to define the first oligonucleotide primer in section i) to comprise a sequence complementary to a 15-30 bp segment of DNA between positions 5124-5524 of the MCOLN1 gene, and to define the second oligonucleotide primer in section ii) to comprises a sequence complementary to a 15-30 bp

segment of DNA between positions 5541-5941 of the MCOLN1 gene. Thus Applicant respectfully requests reconsideration and withdrawal of the rejection.

Rejections under 35 U.S.C. § 103

Rejection under 35 U.S.C. § 103(a) over Edelmann, et al. as evidenced by GenBank AF287270 (2000) in view of Doll, et al.

The rejection of claims 19, 23-25, 28 and 32-34 as allegedly being obvious over Edelmann, et al. (Am. J. Hum. Genet., 2002, vol. 70, p. 1023-27) as evidenced by GenBank AF287270 (2000) and in view of Doll, et al. (Analytical Biochem., 2002, vol. 301, p. 328-32) is respectfully traversed.

To establish a prima facie case of obviousness, three criteria must be met; (1) there must be some motivation or suggestion, either in the cited publications or in knowledge available to one skilled in the art, to modify or combine the cited publications; (2) there must be a reasonable expectation of success in combining the publications to achieve the claimed invention; and (3) the publications must teach or suggest all of the claim limitations. *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991); MPEP § 2142.

Rebuttal to the obviousness rejection of claims 19, 23 and 24

Claims 19, 23 and 24 are directed in general to a method of detecting a large deletion mutation (~6.4 kb) using real-time PCR with fluorescently labeled probes. The assay requires i) a first oligonucleotide primer that comprises a sequence complementary to a 15-30 bp segment of DNA between positions 100-500 of the MCOLN1 gene, ii) a second oligonucleotide primer that comprises a sequence complementary to a 15-30 bp segment of DNA between positions 6956-7356 of the MCOLN1 gene, and iii) an oligonucleotide probe that comprises a sequence complementary to a 13-30 bp segment of a fragment that is amplified using the first and second primer.

Edelmann is directed to a two-step genotyping method for the MCOLN1 gene which requires an initial traditional PCR amplification followed by secondary single probe radioactive

hybridization reactions for determining whether a particular sequence was amplified in the initial reaction. Edelmann does not disclose all the claim elements because it fails to disclose MCOLN1 mutant sequence determination in a single reaction using real time PCR.

Doll is directed to genotyping base pair mutations of the N-acetyltransferase gene using real time PCR. Doll describes a standard two probe method for detecting a single base pair mutation and a three probe system for detecting two base pair mutations located close together (i.e., 1 or 7 base pairs apart). The three probe method is needed because the mutations are too close together for the conventional two probe method.

Applicants respectfully submit that the motivation to combine Edelmann with Doll is lacking because not all primer pairs and probes that might work acceptably in standard PCR will necessarily work for real time PCR because of the more stringent requirements of real time PCR. Sun Declaration ¶ 6.

Furthermore, Doll also does not teach or suggest a method of detecting mutations other than single base pair mutations using real time amplification. Doll does not address how to genotype different a large deletion as required by claims 19, 23 and 24.

The GenBank sequence is unable to cure the deficiencies of Edelmann and/or Doll because the entry merely provides the sequence of the gene and contributes nothing to how to detect the presence of a mutant MCOLN1 sequence.

Moreover, the design strategies for a probe which detects a large deletion mutation as required by the claims differ for that of single base mutations as described by Edelmann. The third probe of Edelmann can only be used for the exact deletion mutation of nucleotides 511 to 6943 of the MCOLN1 gene because the Edelmann probe binds nucleotides 504 to 510 linked directly to nucleotides 6944 to 6954. Edelmann, p. 1024, col. 2, lines 19-20. In contrast, the third probe of the present invention allows for detection of various deletion mutations because it is not dependent on exact splicing.

Applicants respectfully submit that even if there was motivation to combine the references there would be no reasonable expectation of success because neither Doll nor the GenBank accession teach or suggest a way to address the difficulties of adapting MCOLN1 genotyping to real time PCR.

Thus, because the cited references alone or in combination fail to provide a prima facie case of obviousness, Applicant respectfully requests reconsideration and withdrawal of the rejection.

Rebuttal to the obviousness rejection of claims 25, 28 and 32-34

Claims 25, 28 and 32-34 are directed to methods of genotyping two types of mutations, a single base pair mutation and a large deletion mutation in the MCOLN1 gene in a single multiplex real-time amplification reaction using two primer pairs and three probes to distinguish the two different amplicons generated by the amplification reaction.

Edelmann clearly fails to disclose genotyping multiple mutations of the MCOLN1 gene using real time PCR so the Examiner apparently turns to Doll to address the gap. Office Action p. 10, lines 6-10. However, Doll merely teaches genotyping using real time PCR of an unrelated gene and says nothing about how to achieve detection of both a single base mutation and a deletion mutation in the MCOLN1 gene in a single real time PCR assay as required by claims 25, 28 and 32-34.

Thus, the failure of the combination of Edelmann, Doll, and the GenBank sequence AF287270 for teaching or suggesting the genotyping of a deletion mutation in the MCOLN1 gene applies similarly to claims 19, 23 and 24. The references are further deficient in teaching or suggesting how to detect both a single base mutation and a deletion mutation in the MCOLN1 gene as required by claims 25, 28 and 32-34.

Motivation to combine would be similarly lacking because Doll does not teach any general principles as how to apply a real time system to a multiplex amplification assay such as Edelmann. Doll only teaches how to detect two mutations simultaneously from an amplicon

generated from a single primer pair and does not address detecting two mutations from amplicons generated from two primer pairs as required by the instant claims. *See* Doll, p. 330, col. 2, lines 3-8.

A reasonable expectation of success also would be lacking for the combination because Doll does not teach or suggest a method of detecting mutations other than base pair mutations by real time amplification. Doll is only able to detect two mutations in one amplification reaction because the mutations are both base pair mutations and the two mutations are only 1 or 7 base pairs apart. Doll does not teach or suggest how to detect two different types of mutations, such as a base pair mutation and a deletion mutation, in the same reaction. Thus, there would be no reasonable expectation of success in modifying Doll to achieve a more complex two product format from a single amplification reaction.

Thus, because the cited references alone or in combination fail to provide a prima facie case of obviousness, Applicant respectfully requests reconsideration and withdrawal of the rejection.

Rejection under 35 U.S.C. § 103(a) over Edelmann, et al. in view of Doll, et al. and further in view of GenBank AF287270 and Buck, et al.

The rejection of claims 29-32 as allegedly being obvious over Edelmann, et al. in view of Doll, et al. and further in view of GenBank AF287270 and Buck, et al. is respectfully traversed.

As discussed in detail above, Edelmann, Doll and GenBank AF287270 fail to provide a prima facie case of obviousness. As acknowledged by the Examiner, Edelmann and Doll further fail to disclose the real time amplification primer and probe sequences of claims 29-32. Office Action, p. 12, lines 13-17. For this, the Examiner turns to the GenBank AF287270 and Buck. GenBank AF287820 is cited as merely disclosing the MCOLN1 gene sequence. However, Buck is unable to cure the deficiency because Buck merely describes sequencing primers which are unrelated to the present invention. Applicant takes issue with the Examiner's assertion that any potential primer and/or probe in a given nucleic acid sequence would be functionally equivalent.

In this regard, Applicant submits Bernard, et al., Am. J. Pathology, vol. 153, p. 1055-61 (1998), which notes that "[g]enotyping with multiplexed hybridization probes has technical challenges and limitations in addition to the optimizations often necessary for multiplexing primer sets. For example, the melting temperatures . . . [and] unexpected variants may cause erroneous interpretation." Bernard, p. 1060, col. 1, last paragraph to column 2, first full paragraph. Applicant respectfully submits that not all potential sequences can be expected to be functionally equivalent as asserted by the Examiner. Given the increased stringency of real time conditions, there would be an even lower expectation of success adapting primers from a standard PCR assay to a real time assay. *Id.* at ¶ 6. Thus, no prima facie case of obviousness has been established and Applicant respectfully requests reconsideration and withdrawal of the rejection.

CONCLUSION

In view of the above amendments and remarks, reconsideration and favorable action on all claims are respectfully requested. In the event any matters remain to be resolved in view of this communication, the Examiner is encouraged to contact the undersigned so that a prompt disposition of this application can be achieved.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 50-0872. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 50-0872. If any extensions of time are needed for timely acceptance of papers

submitted herewith, Applicants hereby petition for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 50-0872.

Respectfully submitted,

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By _____

FOLEY & LARDNER LLP
Customer Number: 30542
Telephone: (858) 847-6722
Facsimile: (858) 792-6773

Richard J. Warburg, Reg. No. 32,327
By Barry S. Wilson, Reg. No. 39,431
Attorney for Applicant